

Supplementary Material

Ultrasound-Based Elastography

Transient Elastography

TE was the first commercially available ultrasound-based elastography technique developed for the measurement of liver stiffness using a dedicated device (FibroScan, Echosens, Paris, France).¹ TE measures the velocity of a low-frequency (50 Hz) elastic shear wave propagating through the liver. This velocity is directly related to tissue stiffness, called the elastic modulus, expressed as $E = 3\rho v^2$, where v is the shear velocity, and ρ is the density of tissue, assumed to be constant: the stiffer the tissue, the faster the shear wave propagates. The examination is performed on the right lobe of the liver through the intercostal space. The measurement depth is between 25 and 65 mm using the M-probe (standard probe) and between 35 and 75 mm using the XL-probe. As suggested by the manufacturer, 10 successful acquisitions should be performed on each patient. The median of these measurements is displayed and used for interpretation. Results are expressed in kPa, and range from 2 to 75 kPa with a normal value around 5 kPa.²

Advantages of TE include a short procedure time (<5 minutes), immediate results, good reproducibility, and the ability to perform the test at the bedside or in an outpatient clinic—it is not a difficult procedure to learn. Therefore, TE can be considered as a point-of-care technique (Table 2). However, accurate results require careful interpretation of data, based on at least 10 validated measurements, a success rate (the ratio of valid measurements to the total number of measurement) >60%, and an interquartile range (IQR; reflects variations among measurements) of <30% of the median value (IQR/LSM ≤30%).³ It has been suggested that an even lower IQR should be used, especially in non-Asian patients with advanced fibrosis, but these criteria have not been independently validated.⁴

The major challenge for the use of TE in NAFLD patients in clinical practice is the high rate of failure (no valid shot) or unreliable results (not meeting manufacturer's recommendations: ie, valid shots <10, success rate <60%, or IQR/LSM >30%) in these patients, ranging from 11% to 26.8% using M-probe (Table 4). Such a wide range may be explained by the differences in the definitions used (failure vs unreliable results), as well as in the BMI of the studied populations (higher rates in the populations with higher BMI). In the largest series to date, on more than 13,000 examinations in 7261 European patients with chronic liver disease seen during a 5-year period, failure to obtain any measurement was observed in 3% of cases and unreliable results (not meeting manufacturer's recommendations) in 16%,⁵ mostly due to patient obesity or limited operator experience. The XL-probe has been proposed by the manufacturer to overcome these limitations for overweight and obese patients.⁶ The software of the system controls the choice of the probe based on the skin-to-liver capsule distance. Generally, the XL-probe is chosen when this distance is >25 mm. In a recent multicenter US study, based on 1696 examinations in 992 NAFLD patients ($BMI\ 33.6 \pm 6.5\ kg/m^2$) in whom both M- and XL-probes were

used, the failure and unreliable results rate were 3.2% and 3.9%, respectively.⁷ Importantly the liver stiffness cutoffs are lower with the XL-probe than with the M-probe by around 1.5 kPa and need to be adapted.⁸

The risk of overestimating liver stiffness values has been reported with other confounding factors, including ALT flares,^{9–11} extrahepatic cholestasis,¹² congestive heart failure,¹³ and, more recently, food intake.^{14–16} Therefore, TE should be performed in patients fasting for at least 2 hours.¹⁷ The influence of steatosis in NAFLD patients is still a matter of debate, with conflicting results: some studies suggest that steatosis is associated with an increase in liver stiffness,^{18,19} whereas others do not.^{20–22}

Controlled Attenuation Parameter

Recently, a novel parameter, the CAP has been proposed for the noninvasive grading of hepatic steatosis using TE (FibroScan 502 Touch; Echosens, Paris, France). CAP measures the degree of ultrasound attenuation by hepatic fat at the same time, on the same volume, and on the same signal as liver stiffness is measured at the center frequency of the FibroScan M-probe (3.5 MHz).²³ Results are expressed as decibels per meter (dB/m) and range from 100 to 400 dB/m. CAP has been initially only available with the M-probe, but has recently become available also with the XL-probe.²⁴ CAP has been shown to have good inter-observer reproducibility with concordance between observers ranging from 0.82 (95% CI, 0.78–0.85)²⁵ to 0.84 (95% CI, 0.77–0.88).²⁶ However, the agreement between raters decreased for $BMI >30\ kg/m^2$ (0.65) and for CAP values <240 dB/m (0.44).²⁵ Intra-observer reproducibility remains to be studied.

The influence of food intake on CAP results has been examined by 2 studies with conflicting results: one²⁷ showed an increase in CAP values by 10%, whereas another²⁸ observed a significant decrease. Additional data are needed before any firm conclusion can be drawn.

Failure rate of obtaining any CAP measurement in patients with NAFLD, using the M-probe, ranges from 0% to 24% (Table 3). Interestingly, in a large study²⁹ based on 5323 examinations performed with M-probe in 4451 patients with chronic liver disease, failure ranged from 0.5% in young males with no sign of the metabolic syndrome to 33% in elderly females with diabetes and hypertension. Failure was significantly associated with older age, BMI, presence of metabolic syndrome, and with female sex, a finding consistent with what has been reported for LSM.⁵ However, when using the XL-probe, the failure rate was lower at 3.2%.⁷

In the absence of specific quality criteria provided by the manufacturer, investigators have used those recommended for LSM. Recently, an international multicenter study, including 754 consecutive patients, in whom CAP was measured by the M-probe before liver biopsy, has shown that the accuracy of CAP declined when its IQR was >40 dB/m with AUROC for fatty liver of 0.77 vs 0.90, in patients with IQR <40 dB/m ($P = .004$).³⁰ The authors suggested that an IQR <40 dB/m could be used as a quality criterion. Other authors suggested IQR <30 dB/m for a valid CAP.³¹ These findings require further external validation before any recommendation can be made.

In summary, recent guidelines recommend that TE should be performed using a standardized protocol in fasting patients with critical interpretation of results taking confounding factors into account.^{17,32,33}

Point Shear Wave Elastography

pSWE, which includes ARFI, is based on the measurement of shear waves and has the advantage over TE that it is integrated in conventional ultrasound systems³⁴ (Table 2). This enables pSWE in addition to abdominal ultrasound scan with the same system, which is of special interest in countries where the same operator can perform conventional ultrasound and pSWE. At present, most large ultrasound companies offer an additional elastography method integrated in their ultrasound systems. Region of interest (ROI) localization can be chosen under B-mode visualization. A single acoustic impulse is used to induce a shear wave within a small ROI (approximately 1.0×0.5 cm) and the velocity of shear waves is measured in meter/s or kPa. The technique for shear wave induction, the frequencies used, as well as the size of ROI differ between companies and need be taken into account when interpreting the results.^{17,32,33} Measurement should be performed 1–3 cm below the liver capsule. A median of 10 measurements should be used for clinical interpretation. Improved quality is obtained by pSWE estimation algorithms, which warn the user if measurement is not adequate. In addition, quality criteria, such as an IQR/median $\leq 30\%$ and SD $\leq 30\%$ have been reported to improve accuracy.³² pSWE can be performed in patients with obesity and ascites. A disadvantage is the smaller size of ROI compared to TE and the less-evaluated quality criteria. Like TE, pSWE values increase after meal intake. Elastography should therefore be performed after fasting for at least 2 hours.³²

2-Dimensional Shear Wave Elastography

2D-SWE is the most novel ultrasound-based elastography method.³⁴ Like pSWE, it is integrated in conventional ultrasonography systems, enabling the additional performance of elastography with the same probes as abdominal ultrasound (Table 2). Multiple shear waves are induced using acoustic impulses. The size of the ROI of interest can be increased to approximately 2×2 cm and shown as either single image or in real-time. Velocity of stiffness can then be measured at varying locations within this ROI and statistical quantities, such as the mean, SD, minimum and maximum values of the 2D-SWE or Young's modulus in kPa are calculated and displayed. The measurement box should be placed at least 10 mm below the liver capsule. At least 3 measurements should be obtained and the report should include median and IQR. The technique for shear wave induction, as well as the frequencies used, differ between companies and should be taken into account when interpreting the results. All 2D-SWE systems have some kind of quality indicators of the shear wave speed estimate. However, quality criteria for clinical interpretation remain poorly evaluated.^{17,32,33} It can be performed in patients with obesity and ascites. Like TE, 2D-SWE values increase

after meal intake. Elastography should therefore be performed after fasting for at least 2 hours.³²

Magnetic Resonance-Based Imaging

Over the last 2 decades, the utility of MRI-based methods have evolved to their current state and positioned themselves as one of the most accurate methods to noninvasively quantify liver fat, liver iron, and liver fibrosis (Table 2). Two key quantitative biomarkers that will be discussed in more details include MRI-PDFF and MRE.^{35,36} Magnitude-based gradient-recalled-echo technique estimates PDFF, an MRI-based biomarker of liver fat content^{37–39} using low flip angle to minimize T₁ bias^{38,40,41} and 6 gradient-recalled echoes to calculate and correct for T₂* signal decay.^{38,40–42} Parametric liver PDFF maps are computed pixel-by-pixel from source images using custom-developed software that models observed signal as a function of echo time (TE), taking into account the multiple frequency components of triglyceride.^{40,41,43–45} PDFF values are obtained by placing ROIs in representative portions of the maps. The rationale for using MRE to assess NASH and fibrosis is that extracellular matrix alterations associated with these conditions harden the liver and change its mechanical properties.^{46,47} Currently, 2D-MRE is available clinically. In this section, both MRI-PDFF and 2D-MRE will be discussed as the lead MRI biomarkers and we will briefly discuss some of the emerging MRI based biomarkers as well.

Magnetic Resonance Imaging Proton Density Fat Fraction

MRI-PDFF is an emerging accurate, reproducible, quantitative imaging-based biomarkers that has the ability to estimate the quantify liver fat in its entire dynamic range.³⁶ MRI-PDFF improves upon previous methods for fat quantification as it corrects for T₁ decay and T₂* and is unaffected by the age, sex, body mass index, iron content, inflammation, and fibrosis.³¹ MRI-PDFF of the liver is defined as the ratio of the density of mobile protons from triglycerides in the liver and the total density of protons from mobile triglycerides and mobile water.⁴⁸ There are 2 methods to estimate MRI-PDFF, including the magnitude MRI-PDFF or ideal MRI-PDFF, which have a high degree of correlation between them and either method can be used to assess liver fat.⁴⁸ Both methods provide rapid assessment of liver fat in all the 9 segments of the liver within a short breath hold (approximately 20 seconds).³¹ Reproducibility of PDFF measurements might be affected by food intake,⁴⁹ but more data are needed before any firm conclusion can be drawn. Among all methods for detection of liver fat and quantification of liver fat MRS-PDFF and MRI-PDFF have emerged as the gold standard. MRI-PDFF has been validated in numerous studies comparing against MRS, phantom studies, ex vivo human liver tissue, as well as liver histology, in patients with and without NAFLD.^{50,51}

Magnetic Resonance Elastography

MRE is an imaging technique that measures the stiffness of the liver by introducing shear waves and imaging their

propagation using MREs.⁵² MRE (Resoundant, Rochester, MN) requires a special adaptation and proprietary hardware and software installment over conventional MRI scanners. During an MRE, shear waves at 60 Hz are mechanically generated by a circular device that is attached to the right side of the chest wall anterior to the liver. These shear waves and their propagation are then visualized via a 2D gradient-recalled echo pulse sequence. Wave images are interpreted by generating shear wave elastograms by the software. The mean LSM is a summary of the average per-pixel stiffness measurements from the regions of interest.

MRE has a low failure rate typically 1%–2%. It is not affected by ascites and etiology of liver disease.³⁶ However, when massive, ascites has been recently suggested to be associated with MRE failure in a large retrospective study.⁵³ There is conflicting evidence on the effect of BMI on MRE measurements: a recent study found that BMI was not a contributing factor in failure,⁵⁴ but found waist circumference to be a significant factor of failure whereas another study found that BMI was associated with MRE failure.⁵³ It should be stressed, however, that BMI has an impact for fitting into an MRI scanner not for getting an MRE. Finally, MRE can be affected by iron overload, but this can be assessed during an MRI using R2* as an estimate of iron content in the liver.

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